Remarks

The response that was filed on January 5, 2004 was completely responsive to the Office Action that was mailed on October 3, 2003. This Voluntary Amendment merely cancels currently pending Claims 29-58.

Hereinafter, the claims that are pending prior to the entry of the amendments in this Voluntary Amendment are called "currently pending claims." This Voluntary Amendment cancels currently pending Claims 29-58 without prejudice or disclaimer. Upon amendment, the above-identified U.S. patent application will have one independent claim (currently pending Claim 59) and 32 total claims (currently pending Claims 59-90). The Applicant previously paid for up to three independent claims and up to 62 total claims. Therefore, no fee is due for excess claims.

Applicant submits the following arguments to be entered into the prosecution history.

1. Patentability of the claimed invention in view of Lonnerdal and Shewry

The Examiner cites the following documents in item 2 of the Office Action:

Lonnerdal and Iyer "Lactoferrin: molecular structure and biological function" Ann Rev Nutr. 1995, 15: 93-110; hereinafter called "Lonnerdal".

Shewry et al, "Seed Storage Proteins: Structures and Biosynthesis" The Plant Cell, July 1995, Vol. 7, 945-956; hereinafter called "Shewry".

The Applicant submits that according to Claim 59, the technical feature representing a contribution over the art is now defined as a plant expression cassette, said expression cassette allowing in seed specific expression of non-degraded human lactoferrin.

The Applicant submits that the cassette claimed in claim 59 is not anticipated by

the prior art and is non-obvious over Lonnerdal and Shewry. Applicant further submits that since independent Claim 59 is not anticipated and nonobvious, the dependent Claims 60-90 are not anticipated by and are nonobvious over Lonnerdal and Shewry.

2. Patentability of the claimed invention in view of the art

2.1. Applicant refers to the art as cited in the International Preliminary Examination Examination Report of December 8, 2000. Applicant refers to documents D1-D9 of the Report. The documents D1-D9 are as follows.

D1: SALMON V. ET AL.: 'Production of human lactoferrin in transgenic tobacco plants' PROTEIN EXPRESSION AND PURIFICATION, vol. 13, 1998, pages 127-135; hereinafter called "D1".

D2: MITRA A ET AL: 'EXPRESSION OF A HUMAN LACTOFERRIN CDNA IN TOBACCO CELLS PRODUCES ANTIBACTERIAL PROTEIN(S)' PLANT PHYSIOLOGY,US,AMERICAN SOCIETY OF PLANT PHYSIOLOGISTS, ROCKVILLE, MD, vol. 106, no. 3,1994, page 977-981; hereinafter called "D2".

D3: WARD P P ET AL: 'A SYSTEM FOR PRODUCTION OF COMMERCIAL QUANTITIES OF HUMAN LACTOFERRIN: A BROAD SPECTRUM NATURAL ANTIBIOTIC'BIO/TECHNOLOGY,US,NATURE PUBLISHING CO. NEW YORK, vol. 13,1995, page 498-503; hereinafter called "D3".

D4: ZHANG ET AL: 'DEVELOPMENT OF TRANSGENIC PLANTS WITH NON-PLANT ANTIBACTERIAL PROTEIN GENES FOR RESISTANCE TO BACTERIAL PATHOGENS (LACTOFERRIN, NICOTIANA TABACUM, AGROBACTERIUM TUMEFACIENS) DIALOG DISSERTATION ABSTRACTS,XXXX,1996; hereinafter called "D4".

D5: WO 96 37094 A; hereinafter called "WO'094".

D6: WO 91 13993 A; hereinafter called "WO'993".

D7: WO 98 06860 A; hereinafter called "WO'860".

D8 WATANABE Y ET AL: 'Nucleotide sequence of the basic 7S globulin gene from soybean. 'PLANT PHYSIOLOGY, (1994 JUL) 105 (3) 1019-20; hereinafter called "D8".

D9: FR-A-2 762 850; hereinafter called "FR'850".

2.3. Applicant respectfully submits the following brief review of the state of the art concerning the expression of hLF in plants.

The state of the art teaches that, in most cases, the expression in plant of a full length, non-degraded protein of hLF has been unsuccessful, and even acknowledged by certain authors as an ascertained difficulty. See WO'860.

The disclosures of D2, D4 and WO'094, dated respectively 1994, 1996 and 1995, prove that all attempts of obtaining a full-length (non-degraded) expression of the hLf in plant failed.

The cited documents disclose, in fact, the expression of a 48 KDa fragment of the protein whereas the mature hLF protein is of about 80KDa.

With respect to the achievements disclosed in WO'860 recites as follows: *"Human lactoferrin cDNA was recently expressed in tobacco suspension cells to test the antibacterial activity of lactoferrin in plants. Transgenic calli produced a single peptide of 48 kD, which is significantly smaller than the full-length lactoferrin protein. Total protein extracts made from transgenic tobacco callus exhibited much higher antibacterial activity than commercially available purified lactoferrin as determined by the decrease of colony forming units when tested with four phytopathogenic species of bacteria. The full-length lactoferrin was never detected in transgenic calli implying that the lactoferrin gene product had undergone posttranslational processing. Since a human lactoferrin gene was expressed in Aspergillus nidulans with the production of large amounts of full-length lactoferrin, it was theorized that full length-plant produced lactoferrin does not undergo proper folding and the unfolded part is degraded."*

2.4. The applicant submits that the Claims 59-90 are not anticipated by the cited documents for the following reasons:

The present application discloses an in-seed specific expression of undegraded human Lactoferrin.

Said expression is achieved through the construction of expression cassettes comprising regulatory sequences derived from β-conglycinin or soybean basic

globulin 7S and a DNA sequence coding for human Lactoferrin, said expression cassettes allowing the specific in seed expression of an undegraded recombinant hLf having a size and a degree of glycosilation comparable to that of the native hLf. The application also discloses vectors comprising said cassette, transformed plant cells and cell aggregates comprising said vectors, transgenic plants and seeds being capable of expressing an undegraded recombinant hLf and uses thereof.

D2 and D4 disclose the in plant expression of a 48KDa fragment of hLf using the hLf cDNA under the control of the 35S CaMV promoter.

WO'094 also discloses a partial expression of a full length hLf always in presence of degraded fragments of the protein of 38 or 42 KDa (as acknowledged by the authors, see WO'094 page 9 lines 34 – 37, page 11, lines 1 and 2, and Figure 6) only in leaf with the 35S CaMV promoter (see page 8, line 9, page 9, line 1).

Hence, Claim 59 is not anticipated by D2, D4 and WO'094.

D3 discloses the expression of hLF in fungi as a glucoamylase fusion protein; hence, Claim 59 is not anticipated by D3.

WO'860 discloses the plant expression of a fragment of bovineLf, the bovine Lactoferricin, said fragment DNA sequence being optimized for the in plant expression, the suitable promoters for said expression, according to the description, are listed in page 4, lines 27-34, and are: CaMV 19S or 35S promoters, nopalyne synthase promoters, pathogenesis-related (PR) protein promoters, rice actin promoter, ubiquitine promoters and Pr-1 promoters from tobacco, Arabidopsis or maize.

Hence, claim 59 of the present application is not anticipated by WO'860.

Lommerdal is a general review concerning Lactoferrin. In the first paragraph of page 98, mention of recombinant human Lf is made. Reference is made in Lommerdal to the expression of hLf in baby hamster kidney cells, feasibility of

large scale hLf for clinical trials using transgenic animals, production of large scale hLf in micro organisms such *Saccharomyces* or *Aspergillus*. Therefore, Lommerdal does not disclose any in plant expression of hLf and Claim 59 is hence not anticipated by Lommerdal.

Shewry is a paper concerning seed storage proteins and does neither disclose an in plant expression of hLf nor of heterologous genes or plant expression cassettes for targeted in-seed expression, hence, Claim 59 is not anticipated by Shewry.

D8, although titled "Nucleotide sequence of the Basic 7S Globulin Gene from Soybean" D8 does not disclose said nucleotide sequence. On the contrary, it clearly states (see page 1019, column 1, lines 15-17): "However the genomic DNA encoding Bg has not beencharacterized so far in any organism".

Following said sentence, the article states that the nucleotide sequence of Bg gene was determined without disclosing it. No in-plant expression of hLf is disclosed in D8; hence Claim 59 is not anticipated by D8.

FR'850 discloses the expression of full length Lf in plant corresponding to the scientific publication D1.

The promoters suitable for the in plant expression according to FR'850 are listed in page 8, lines 32-37 and page 9, lines 1-14. Said promoters are: the CaMV 35S promoter or the double CaMV 35S promoter, the PCRU promoter of the radish cruciferine gene, the promoters PGEA1 and PGEA6, the super-promoter PSP, the rice actin promoter linked to the actin rice intron (PAR-IAR), and the barely High Molecular Weight Glutenine (HMGW) promoter.

The suitable signal peptide (leader sequences) indicated is listed in page 9, lines 24-37and page 10, lines 1-11.

Said signal peptides are: the sweet potato sporamine A signal peptide and derivatives thereof.

Hence, claim 59 is not anticipated by FR'850.

D1, which is the subsequent scientific publication corresponding to document FR'850, discloses the expression of Human Lactoferrin (hLf) in tobacco leaves (see page 128, column 2, protein extraction) using constructs containing either the native signal peptide or the sweet potato sporamin (PSSp) fused to human lactoferrin encoding cDNA under the control of the enhanced 35S promoter and 35S terminator of the cauliflower mosaic virus (CaMV). The expression is obtained in *Nicotiana tabacum xanthi NC*.

Hence, claim 59 of the present application is not anticipated by D1.

WO'993, which the Applicant and the IPEA consider as the closest cited art, is an international application disclosing a seed specific expression cassette for the expression of high sulphur seed storage proteins and a non-plant gene, the bovine somatotropin (Bst) (see page 7, lines 34-37).

A single reference to legumes globulins describing the two types of globulins (the glycosylated 11 S proteins and the glycosylated 7 S fraction) is made in the background of the claimed invention in page 1, lines 22-25 of WO'993.

WO'993 discloses a "seed specific expression cassette which has a promoter derived from either phaseolin, α' subunit of β-conglycinin or β-zein 15Kd, a translation initiation signal from either phaseolin, α' subunit of β-conglycinin or β-zein 15Kd or an animal gene, a gene derived from brazil-nut sulphur-rich seed storage protein or an animal gene, a translation termination region derived from either the animal gene, phaseolin or β-zein 15Kd, and at least one polyadenilation region from phaseolin, the animal gene or β-zein 15Kd; wherein the regulatory sequences are operatively linked to one another in such a manner that the gene is expressed in seed or seed storage protein bodies" (see page 5 lines 11-21).

WO'993 does not disclose the use, in the disclosed expression cassettes, of other suitable promoters or signal sequences, besides the above indicated ones, for the in seed specific expression.

No mention is made, throughout all the description, of the preferred embodiments and the examples, to soybean basic globulin 7S. No indication of the basic globulin 7S sequence is given, and, consequently no indication of any regulatory sequence of said protein is given.

The document does not enable the person skilled in the art for the use of 7S Bg sequences in the construction of in seed specific expression cassettes.

Moreover, although generally mentioning "an animal gene" WO'993 only discloses and enables the expression of bovine somatotropin (see whole document and, in particular, examples 9 and 10 and claims). Hence, the document does not disclose the expression of Lactoferrin in general, of human lactoferrin in particular. Hence, although WO'993 discloses an expression cassette comprising β-conglycinin regulation elements, Claim 59 is not anticipated by WO'993.

2.5. The applicant submits that the Claims 59-90 are non-obvious over the cited documents for the following reasons:

FR'850 and D1 disclose an in-plant, more specifically an in-leaf expression of full-length, undegraded, hLf.

In fact, although FR'850 indicates possible expression cassettes introduced in suitable vectors for the in-seed expression in Mais, (see examples 7 and 9, pages 15-17) no examples, results, or any proof of the successful expression of hLf in seed with said cassettes are disclosed or reported in the document. Transgenic Mais plants (i.e. comprising the vector with the heterologous gene) are described, however, all the examples related to the hLf expression in the transformed plants, are only disclosing an expression tested in leaf (see example 14, page 22) with plants transformed with the constructs pBIOC21-PSLf-Lf and pBIOC21-PPSp-Lf in which, as clear from examples 4 to 6 (pages 14 and 15), the Lf gene is under the control of the CaMV double promoter 35S.

Although there are alleged promoters for the in seed expression in the

description of FR'850, i.e. the rice actin promoter and the Mais β-zeine promoter, respectively exemplified in examples 5 page 15 and 9 page 16, expression of hLf is supported in FR'850 only with regards to the in leaf expression with the 35S promoter (see example 14, page 22).

Furthermore, it is to be noted that the subsequent scientific publication of the disclosures of FR'850, which is D1, published one year after the filing date of FR'850, does not even mention the plasmids for the expression in Mais seeds but focuses only on the plasmids wherein the hLf gene is under the control of the 35S promoter and, again, as in FR'850, the protein extraction is made in leaf (see D1, page 128, column 2 "protein extraction".

The fact that the authors have demonstrated neither in FR'850 nor in D1 the hLf expression in seed, together with the facts that the subject of a possible in seed expression as disclosed in FR'850 has been dropped in the disclosures of D1 and that, apparently (at least from a quick search in Medline) the authors have never mentioned again an in seed expression, is an evident proof that the teaching of FR'850 concerning the in-seed expression was largely speculative.

Due to the general inability of expressing lactoferrin in plants in the state of the art, as clear form D2, D4, and WO'860 it is believed that, the person skilled in the art, would find no suggestion that some of the cassettes disclosed in WO'993 would have led to an in-seed expression of undegraded Lactoferrin taking into account the teachings of FR'850 or D1.

In fact, FR'850 and D1 disclose expression systems that are completely different form the ones disclosed in WO'993.

Moreover, even according to the authors of FR'850 and D1, the reason of the achievement of a full-length expression of hLf in plant cannot be explained.

In fact, D1 clearly recites in page 132, column 2, lines 4-16: *"This result is in contrast to work of Mitra and Zhang (D2) who described the isolation of a 48-kDa truncated Lf fragment from transgenic tobacco cells transformed with a 35S promoter-*

hLf construct. Since the authors give no further indications about the nature of the truncated Lf peptide, it is difficult to judge whether the discrepancy with our results is related to the particular construct or linked to the use of different tobacco varieties. Since our aim is to produce in transgenic plants recombinant human Lf for pharmaceutical or nutritional applications, it was crucial to obtain a correctly processed full-length protein."

Therefore, even according to the authors of FR'850 and D1, there is no indication of why and how the system disclosed in said documents could result in a full-length in plant expression of Lf.

The person skilled in the art would hence have no indications on the possibility of effective expression of Lf using an expression cassette of WO'993 or a different seed specific expression cassette.

As no demonstration of an effective in seed expression of undegraded hLf was ever made available to the public at the date of filing, the person skilled in the art would have had no indications that an in-seed expression system as disclosed in WO'993 or even of a totally new one, would have led an effective in-seed expression of undegraded hLf.

Actually, due to the various problems related to the degradation of the in-plant expressed hLf reported in the previous publications and the lack of scientific explanations of the experimental reasons of the only successful trial reported, the person skilled in the art would have expected, when attempting to use a system disclosed in WO'993 or a different in-seed expression system for hLf, to encounter possible folding problems and the following degradation problems known for the in plant hLf expressed protein.

Furthermore, it can even be assumed that the lack of effective results regarding an in seed expression in FR'850 or D1 would have suggested to the skilled person that an in seed expression had not been achieved and certainly would have not prompted said skilled person to use in-seed expression cassettes for the expression in plant of Lf. The apparently satisfactory results obtained with the

35S promoter of FR'850 - D1 would have even taught away the skilled person from using different promoters and from an attempt of an in-seed specific expression.

In view of the above, the applicant believes that the present invention, as claimed in currently pending Claims 59-90, should be regarded as not anticipated by and nonobvious over the cited documents.

This Voluntary Amendment cancels currently pending Claims 29-58 without prejudice or disclaimer. The amendments that are described in the preceding sentence were done to improve the language of the claims and were not done to overcome the prior art, to overcome rejections under 35 U.S.C. § 112, or to overcome any other rejections or objections. The amendments that are described in the first sentence of this paragraph shall not be considered necessary to overcome the prior art, shall not be considered necessary to overcome rejections under 35 U.S.C. § 112, and shall not be considered necessary to overcome any other rejections or objections.

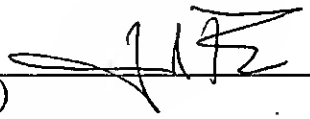
The Applicant reserves the right to seek protection for any unclaimed subject matter either subsequently in the prosecution of the present case or in a divisional or continuation application.

The Commissioner is authorized to charge any additional fees which may be required or credit overpayment to deposit account no. 12-0415. In particular, if this Voluntary Amendment is not timely filed, then the Commissioner is authorized to treat this Voluntary Amendment as including a petition to extend the time period pursuant to 37 CFR 1.136 (a) requesting an extension of time of the number of months necessary to make this Voluntary Amendment timely filed. The petition fee due in connection therewith may be charged to deposit account no. 12-0415.

I hereby certify that this correspondence is being deposited with the United States Post Office with sufficient postage as first class mail in an envelope addressed to Commissioner for Patents, PO Box 1450, Alexandria, VA 22313-1450 on

February 25, 2004
(Date of Deposit)

John Palmer
(Name of Person Signing)



(Signature)

February 25, 2004
(Date)

Respectfully submitted,

John Palmer
Attorney for Applicants
Reg. No. 36,885
LADAS & PARRY
5670 Wilshire Boulevard, Suite 2100
Los Angeles, California 90036
(323) 934-2300